ASD
APOLLO SYSTEMS DEPARTMENT

GENERAL ELECTRIC

NASA CR-
160198

HOUSTON, TEXAS TECHNICAL INFORMATION RELEASE

TIR 741-LSP-8015

FROM	TO
S. N. Brand	J. A. Rummel, Ph. D. /SE2

DATE	WORK ORDER REF:	WORK STATEMENT PARA:	REFERENCE:
7/18/78		NAS9-15487	

SUBJECT A Brief Review of Spaceflight Calcium Metabolism: Results and Methodologies

(NASA-CR-160198) A BRIEF REVIEW OF SPACE FLIGHT CALCIUM METABOLISM: RESULTS AND METHODOLOGIES (General Electric Co.) 24 p
HC A02/MF A01 CSCL 06P

N79-25715

Unclas
G3/52 22187

This report gives a brief review of spaceflight induced osteoporosis, the techniques that were used to detect and measure the osteoporosis, and more sensitive techniques that may be used in the future. Areas of calcium metabolism that may provide insights and direction into the future study of calcium metabolism are also presented.

Susan N. Brand
Susan N. Brand

Attachment
/db

CONCURRENCES

Counterpart.

D. G. Fitzjerrell
Life Sciences Projects Unit Manager: DG Fitzjerrell

C. W. Fulcher
Engrg. & Advanced Programs Subsection Mgr. CW Fulcher

DISTRIBUTION

NASA/JSC:
C. S. Leach, Ph. D.
S. L. Kimzey, Ph. D.
P. C. Rambaut, Sc. D.
C. Sawin, Ph. D.
J. Waligora

GE/TSSD:
S. N. Brand
D. J. Grounds
J. I. Leonard
R. B. Hoffman

ASD 1038

A BRIEF REVIEW OF SPACEFLIGHT CALCIUM METABOLISM: RESULTS AND METHODOLOGIES

The prospect of bone mineral loss has developed into an important and limiting facet of man's adaptation to space. Bone density studies on the crewmen of the early Gemini IV, V, and VII[1] and Apollo VII, VIII[2] space flights showed dramatic degrees of demineralization. These drastic results were not confirmed by the Gemini VII metabolic balance study.[3] Subsequent studies on the crewmen of the longer Skylab 2, 3, and 4 missions consistently showed no bone mineral loss in flights under four weeks, but varying degrees of mineral loss in longer flights.[4,5] The bone mineral losses manifested themselves biochemically as elevated fecal, urinary, and plasma calcium levels over preflight levels and overall negative calcium balances. The urinary calcium levels rose upon exposure to zero-g and plateaued at values of 50 to 100% those of preflight levels. These biochemical analyses collected on the Skylab crews point to early losses of mineral content, with the degree of loss increasing as duration in space increases.[4,5,6,7] Increasing levels of fecal calcium also suggest that factors regulating calcium balance, in particular gastrointestinal absorption, may be selectively insensitive to skeletal rebuilding.[5] The same phenomenon has been observed in bed rested or immobilized subjects[8] and shows no sign of abatement.

Histological evidence of bone demineralization has mainly focused on the use of rats[9,10] and monkeys.[11] In rats the bone demineralization appeared as losses in the number and sizes of trabeculae and in the mass of the metaphyseal spongiosa. No changes were seen in the diaphyses of the tubular bones.[10] Morey and Baylink[9] demonstrated that in rats the mineral loss may be due to a cessation of bone formation. They also presented evidence of a dramatic increase in postflight bone formation to rebuild the demineralized sites in a relatively short period of time. Kazarian and Von Gierke[11] presented a histological relationship between muscle and bone atrophy in immobilized Macaca mulatta Rhesus monkeys. They found that the bone demineralization

resulted in a decrease of the size and number of trabeculae and in the thickness of the cortical bone. Furthermore, enhanced bone resorption was found to be occurring at the site of muscle insertion on the bone and periosteal surfaces resulting in a loss of 30% of the force required to pull the tendon from the bone.[11] This hints at a possible association between muscle tensile and shearing forces and bone maintenance.

Because of the functional interaction between skeletal and muscular tissue, exercise has been suggested as a method to reverse or retard bone demineralization. Few studies have concentrated on the effect of exercise upon bone demineralization though. The bed rest studies by Hulley, et al[12] demonstrated no effect of exercise upon bone demineralization, but the exercises provided particular muscle groups only with a daily regime of movement for 80 minutes a day and with 8 pounds of force. That type of exercise program may have been inadequate. The prevention of bone demineralization has been observed when bed rested subjects interrupted their recumbency by standing quietly for three hours a day[13] or by doing isometric exercises that concentrated 300 to 400 pounds on the os calcis.[13] The Hulley, et al[12] study may have lacked sufficient force, duration, exercise to the proper muscle groups, or any combination of the listed variables.

The effect of exercise, and the association between muscular and skeletal systems in terms of bone maintenance is not understood very well at this time. The design of an exercise program attempting to duplicate the compressional, tensile, and shearing forces applied to the bones by the force of gravity and anti-gravity muscles would be ideal to investigate this problem. Lacking that, exercises involving important muscle groups, of sufficient duration and force are required to properly evaluate their effect upon bone demineralization. Sensitive measurement techniques are also required.

The bone measurements for the space flight studies were achieved by x-ray densitometry[1,2] and gamma ray absorption spectrophotometry.[4]

The gamma ray absorption was determined to be the better of the two techniques, 6.8% versus 7.6% standard error,[14] but not sensitive enough to consistently detect, mineral losses sustained in less than four weeks.[4,5,6] Other techniques including total body neutron activation (TBNA) were not sufficiently sensitive either. With the exception of TBNA, all of the techniques measure total absorption.[14,15,16] Total absorption is the most closely correlated measurement of total mineral content. But space flight osteoporosis appears to occur first in the trabecular bone. The total absorption measurement is not sensitive enough to detect changes in trabecular bone due to overshadowing by the large values of dense cortical bone.[17,18] TBNA acts on the same principle in that it measures total calcium content.[19,20] Consequently, it suffers from the same problem as total absorption and has a standard error of 5.1%.[20] Although radiography is a measure of trabecular bone,[16] it lacks sufficient precision. A technique capable of measuring trabecular bone density is required.

Computed tomography is capable of separating bone by its two different densities, trabecular bone density, and cortical bone density. Total bone mass can also be determined. Figures 1 and 2 demonstrate the ease of the instrument in separating various tissues and dense versus spongy bone. By definition trabecular bone is located at a distance of 10% of the ulna length starting from the styloid process and is measured as 50% of the core of the normal radius. Cortical bone and total mineral content are measured by the absorption of the total cross-sectional area of the radius at 33% of the ulna length from the styloid process (Figure 3). The importance of this bone separation capability is that, in reference to osteoporosis, the sensitivity of trabecular density measurements are five to ten times greater than the sensitivity of total mineral content measurements in related cross-sectional areas of bone.[24] Figure 4 demonstrates the value of trabecular versus total bone density measurements. A group of young adults who had one arm immobilized in a cast for three

ENERGIE (keV)	Fat	Muscle	Water	Plasma	(ρ=1,8-2,1) Compact Bone	Plexi-glas[2]	Reinaly-minium[2]	Hydroxi-apatit[3]	Aluminium-legierung
15,0					11,30-13,19	1,207	21,14		
17,7	0,665	1,152	1,050	1,115					
20,0					5,004-5,838	0,629	9,045		
21,1	0,462	0,759	0,688	0,736					
26,4	0,327	0,485	0,443	0,469					
27,2								8,389	5,776
27,5	0,316	0,455	0,417	0,438				8,156	5,620
30,0					1,724-2,012	0,339	2,916		
31,1	0,276	0,375	0,345	0,360				5,834	4,064
31,7								5,501	3,841
35,5	0,245	0,318	0,295	0,306				4,121	2,911
40,0					0,918-1,071	0,268	1,482		
41,4	0,219	0,274	0,255	0,263					
47,2	0,204	0,248	0,230	0,237					
50,0					0,625-0,729	0,237	0,969		

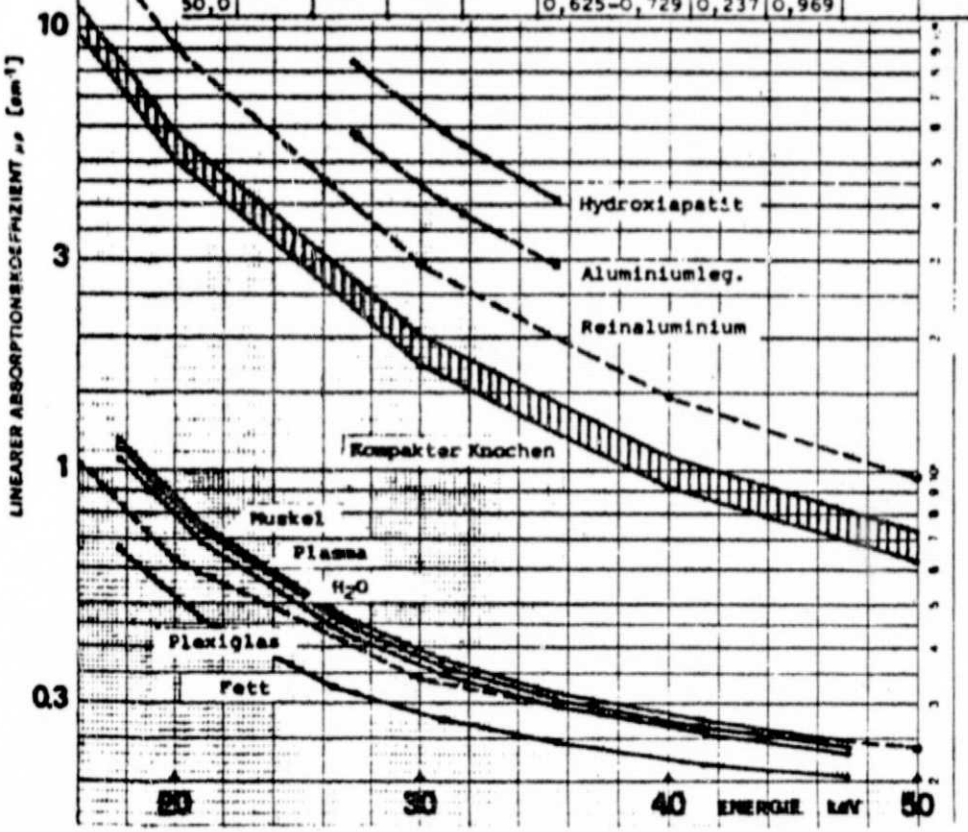

Fig. 1. Absorption Coefficient Differences Between Bone Tissues and Tissue Equivalent Materials. (21)

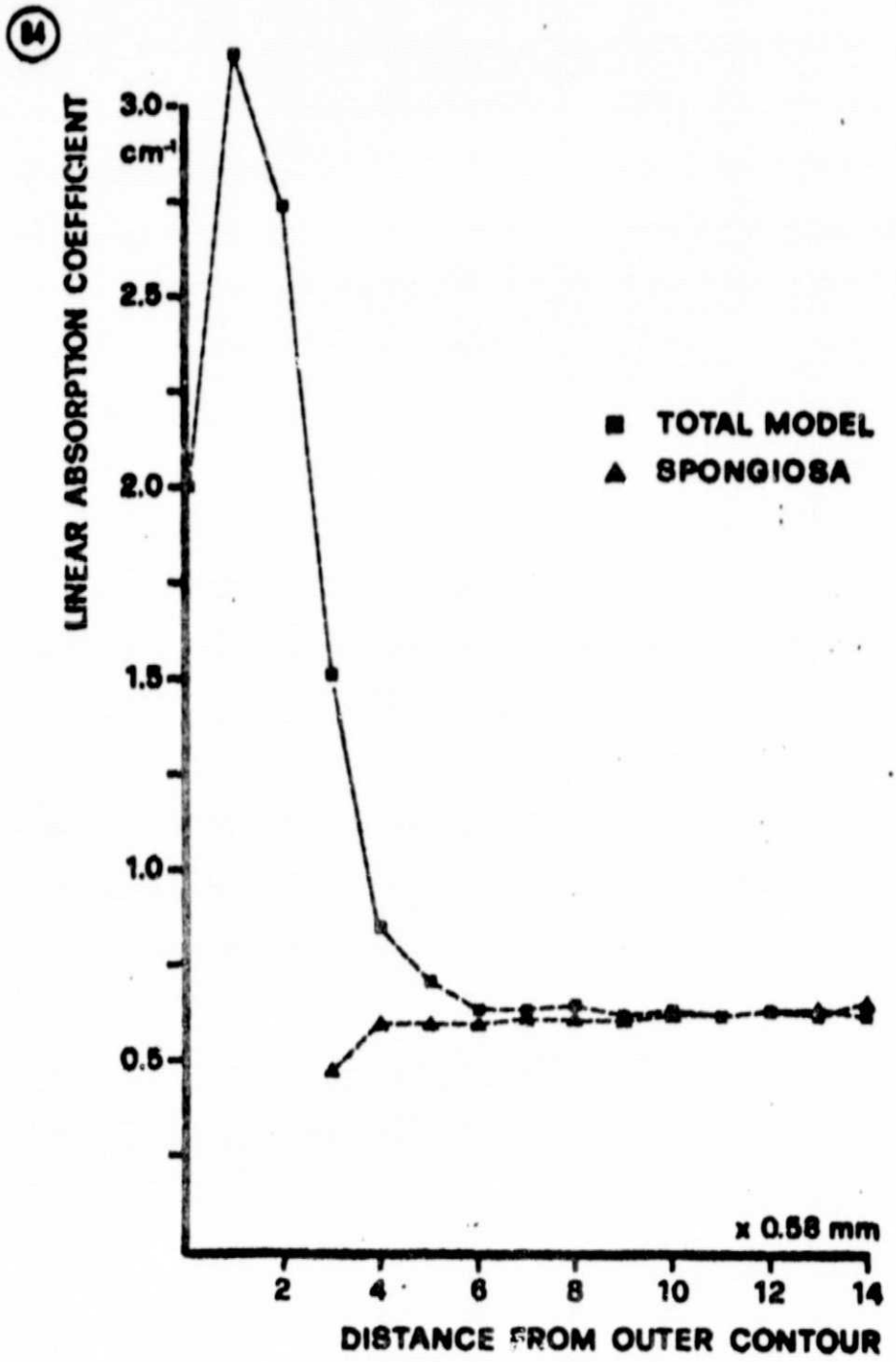

Fig. 2 [21,22] The linear absorption coefficient of a model of bone as the distance into the bone is increased.

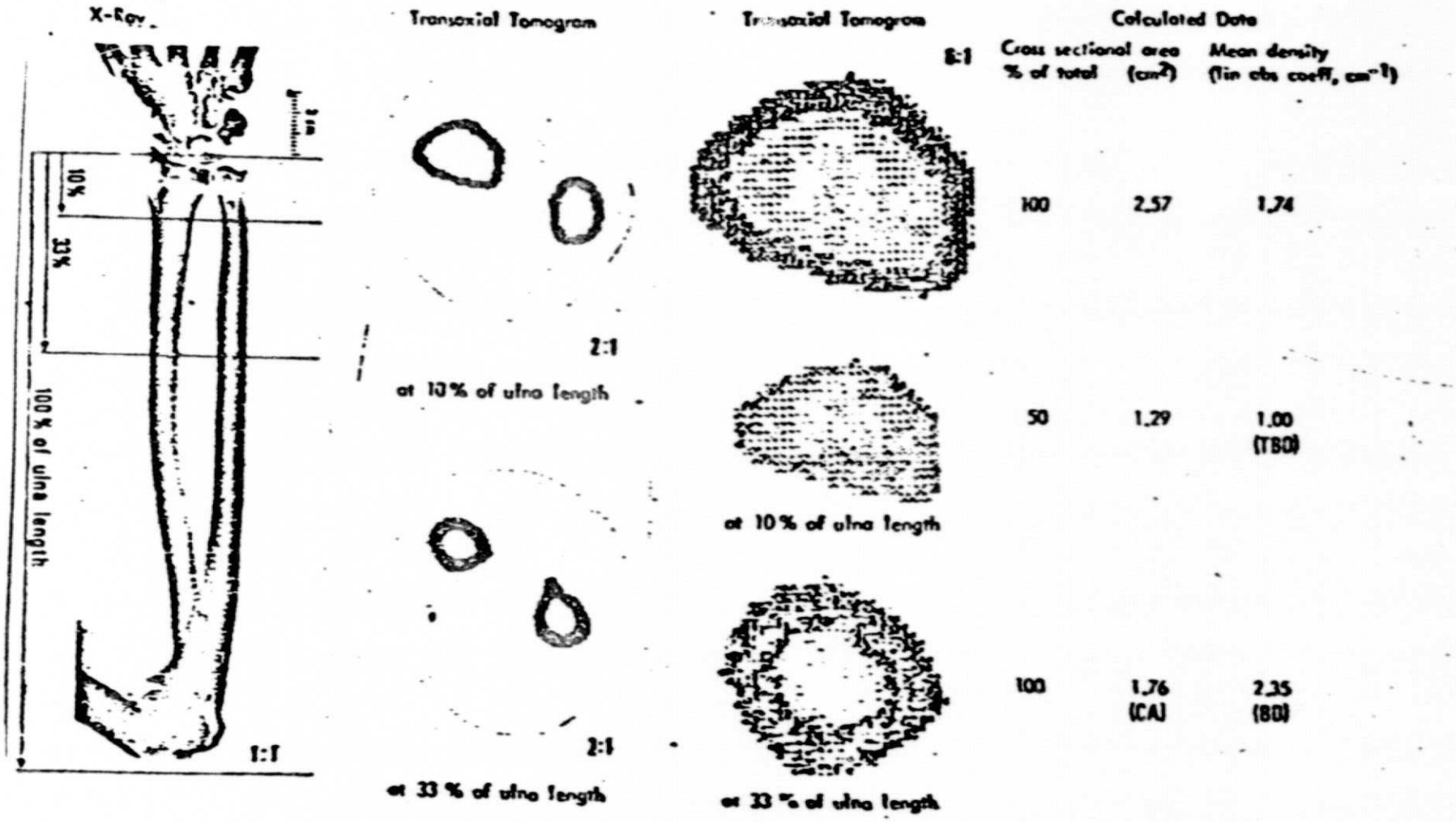

Fig. 3 [23] Tomograms of Trabecular and Cortical Measurements

TBD- and BD-Deviation of Non-Dominant from Dominant (Healthy) and Immobilized from Uninjured Arm (Fracture) at Time of Cast Removal

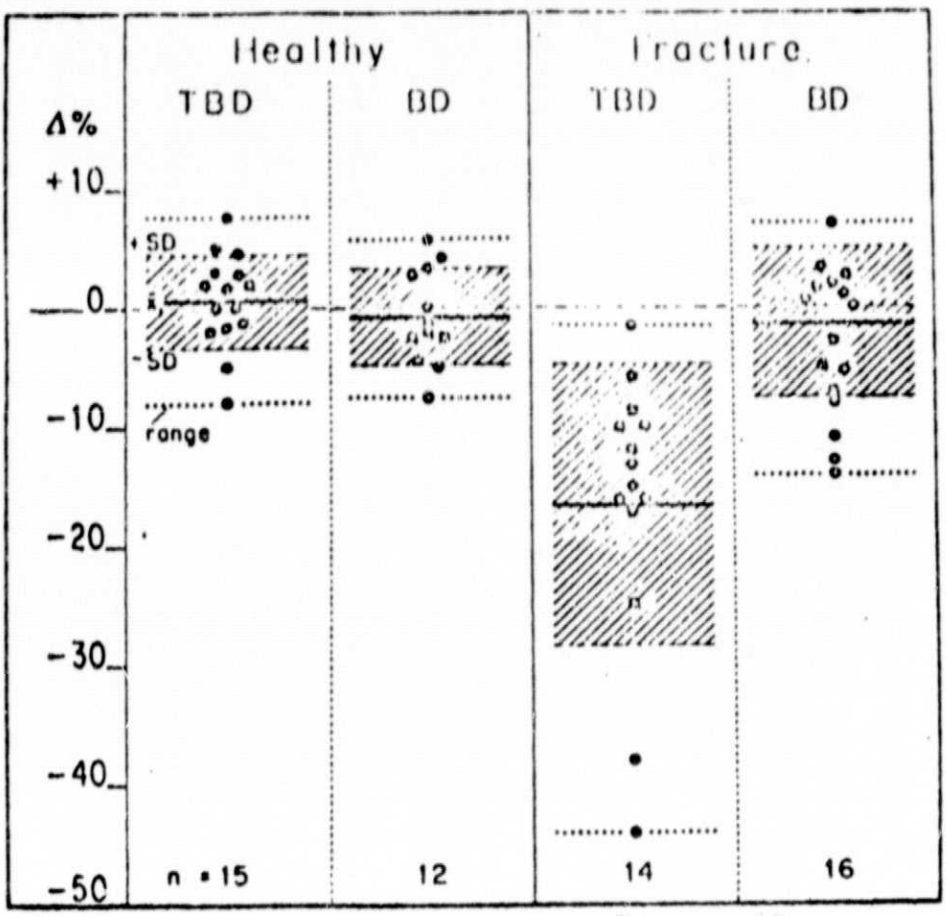

Fig. 4 [25]

weeks showed no changes in total bone density, but an average of 17% change in trabecular bone density.

The technology of the computed tomography is also improved over that of the techniques used in the previous space flights. The x-ray densitometric technique involved a comparison of the density of the sample to the density of a standard, in this case an hydroxyapatite wedge. A roentgenogram was taken of the sample and standard. The roetgenogram was then run through a stationary beam of light. The detector measured the resulting light intensity which was compared to the initial beam intensity. The resulting light intensity of the sample was compared to that derived from the standard precisely quantitating the roentgenogram.[14] The gamma ray absorptiometric technique measured the beam attenuation created when the beam passed through the sample. Each scan was visually represented by 256 points, each point representing 0.5mm of the scan row and inversely related to the relative mineral content. The relative mineral content of each point was expressed mathematically as the log of the ratio of the values of 100% transmission of the beam through water and transmission of the attenuated beam through the bone. This value could be converted to the absorbance of hydroxyapatite by scanning and comparing the values obtained from the calibrated hydroxyapatite wedge. The values of all of the points in each scan row and all of the scan row values were then summated for the total relative mineral content.[14] The bone for this technique was separated from the soft tissue by immersing the sample in water, thus providing a uniform tissue equivalence throughout the scan. Standards consisting of bone embedded in plastic and containing a mineral content similar to that of the bone to be measured, were scanned immediately prior to and following the subject scan.[14]

Computed tomography is a further extension of the gamma ray technique. The width of the sample is linearly scanned during 128 parallel and equally timed intervals. Each interval is called a ray sum and its density value is recorded by a computer. The gamma beam is then rotated 3.75^{o} and the linear scanning

is repeated. This process is carried out 48 times until a semi-circle has been made about the sample. All of the ray sums are processed by the computer to reconstruct a cross sectional image of the sample. The computer uses the convolution method to determine the geometric distribution of the local absorption coefficients[22] which are recorded as a matrix of 128 x 128 elements, or pixels. The pixels are assigned different shades of gray, based upon their magnitude. The reconstruction is then displayed as the color coded representation of the 128 pixels. This allows for the visualization of the mineral distribution. Soft tissue is removed from the determinations of bone mineral content by removing the appropriate density pixels. Trabecular bone is isolated in the same manner, by removing the cortical bone pixels. The advantage of the computed tomography over the x-ray and gamma ray densitometric techniques is that it is capable of distinguishing small differences in gamma ray densities which are too subtle to be detected by the other two methods.

The statistical error of reproducibility in terms of coefficient of variance is $\pm 1\%$ for the trabecular bone measurements, $\pm 2\%$ for the cortical bone and mineral content.[23] Computed tomography is a qualitative determination of mineral content and mineral composition based upon a density distribution. Consequently, no quantitative analysis in terms of mineral loss in hydroxyapatite or calcium units can be given, but a reliable value of mineral density changes can be determined.[17] Spongy bone density can be quantitated to an accuracy of $\pm 2\%$.[22] The machine is easy to use clinically, requires little time, and is the most sensitive instrument to date for the quantitative measurement of trabecular bone changes.

The sensitive measurements derived from computed tomography will only yield density changes in regional areas, specifically the ankle and the wrist. The most popular hypothesis concerning zero-g induced bone demineralization is a dependence of bone maintenance upon stresses induced by gravity and muscular tensile and shearing forces. One approach to studying this hypothesis is

through exercise. Another is to investigate the distribution of demineralization throughout the body. As has been mentioned earlier, Morey and Baylink[9] demonstrated that in rats the mineral loss may be due to a cessation of bone formation and that postflight bone formation increased dramatically to correct the defect in a relatively short period of time. Operating on the idea of a rapid postflight rebuilding of bone, Palmer and Karagianes[26] measured the rate of bone mineral uptake following immobilization with the use of a radioactive bone mineral tracer, strontium 85 (^{85}Sr).

^{85}Sr is a pure gamma emitter with a half-life of 65 days. It is well suited for external counting techniques and is used as a calcium tracer. Calcium isotopes cannot penetrate the tissue depth or have too short of a half-life for adequate study. Strontium has been used as a calcium mimetic to study skeletal metabolism, both for research[27,28] and clinical purposes.[29,30] Strontium is not absorbed by the GIT as well as calcium and is excreted in higher quantities than calcium by the kidney.[31] The result is a significantly lower concentration of strontium in the tissues of the body, especially under oral administration. The strontium that enters the body though appears to have the same plasma kinetics as calcium. To further examine strontium and plasma kinetics, chemical agents modifying the tracer excretion rates were studied.

The removal of radiostrontium from the body immediately upon exposure to the radiostrontium and select chemical agents is basically removal from the blood via the kidneys.[32,33,34] The agents effect the removal at the site of the kidneys itself. The removal of radiostrontium by chemical agents two or more weeks after exposure to the strontium is believed to be removal from the bone.[32,33,34,35] That removal has an identical excretory pattern as calcium. These results indicate that with the exception of the GIT and the kidney, calcium and strontium metabolism are very similar. In actuality, there may be a discrimination at the site of the bone[36,37] as well, but that the discrimination is not so great as to effect plasma concentrations. The discrimination appears to be a function of size differences between the strontium and the

calcium in the crystallization of hydroxyapatite.[36] In the amorphous stage of bone formation strontium is readily incorporated into the bone, but as crystallization occurs, the larger strontium is eliminated and replaced by the calcium. This discrimination is not detrimental to the study of postflight skeletal rebuilding as long as quantitative measurements are not required.

Strontium can be used then as a localizer of spaceflight osteoporosis. Administered immediately postflight, it will be incorporated into the areas of bone undergoing rapid rebuilding. After a one to two week period, those areas and their relative intensities can be identified and categorized. These data will indicate then the primary types of bones affected, the order of magnitude of the effect, and will lead to hypotheses concerning the type of forces influential in the bone demineralization.

The computed tomographic and strontium studies concentrate directly on the metabolism of bones. Balance studies have suggested altered states of calcium metabolism elsewhere also, perhaps at the GIT.[5] It has been suggested that as the duration in space increases, the absorption of calcium from the GIT decreases.[5] Heaney, Saville, and Recker[38] suggest that normal calcium absorption is dependent upon the control of active transport and the uncontrollable process of passive diffusion, a function of intake. An empirical equation which describes calcium absorption (Ca Abs)

$$\text{Ca Abs} = 0.1541 \cdot \text{Ca}_{\text{D}} + 0.3127 \left[\text{Exp}\,(-1.0539 \cdot \text{Ca}_{\text{D}}\right] \cdot \text{Ca}_{\text{D}}$$

where Ca_{D} is dietary calcium, takes both of these factors into account, diffusion by the linear $0.1541 \cdot \text{Ca}_{\text{D}}$, active transport by the exponential function.[38]

Important regulators in the control of the active transport of calcium from the GIT are calcitonin, parathyroid hormone, and metabolites of Vitamin D. They are also important in the promotion of bone resorption and the mineralization of calcium. Altered levels of calcitonin, parathyroid hormone, and Vitamin D have been measured in some of the calcium balance studies, but no significant results have been detected. Differences in Vitamin D metabolites have not been

studied. In view of the tremendous role of Vitamin D and its metabolites[39,40] parathyroid hormone[41] and calcitonin[42] upon calcium metabolism, a disruption of the hormonal system may be seem as a result, or resulting in, a disruption in calcium metabolism. By studying the gastrointestinal absorption rates of calcium at the concentrations of active transport, and by applying tracer studies[43,44] and sensitive radioimmunological assays, differences in the levels of these hormones and their role in the zero-g induced osteoporosis may be established. As examples, Figures 5 and 6 show the relationship between dietary calcium and absorption while Figure 7 is a chromatographic profile of the metabolites of an oral dose of Vitamin D_3 - ^3H in normal, healthy subjects.

Measurements of calcium absorption in man can be done in one of three ways: balance studies, single isotope tracer studies, or double isotope methods. Balance studies are frequently used, but are complicated, demanding, and involve assumptions about calcium bone metabolism which may not be true or hard to maintain under zero-g conditions. These assumptions include a steady-state condition, a constant calcium load in the gut, and that activity, hypothesized to be occurring in the bone, is correct. Tracer methods attempt to alleviate some of the problems typical of balance studies.

Single isotopic methods can be done in one of three ways: tracer injection,[45-48] tracer ingestion,[49,50] or both consecutively.[51] When the tracer is injected into the body, an internal calcium standard is established. The calcium standard is compared to stable calcium levels in the blood and urine. This demands that a precise knowledge of the amount of stable calcium being ingested be known. Levels of the tracer in the feces will yield endogenous calcium secretion rates. If the tracer is administered orally the intake of stable calcium is not important, but a standard value of plasma and urinary calcium values must be presumed. A problem typical of either single isotope method is that it must also include a balance study, thereby making it subject

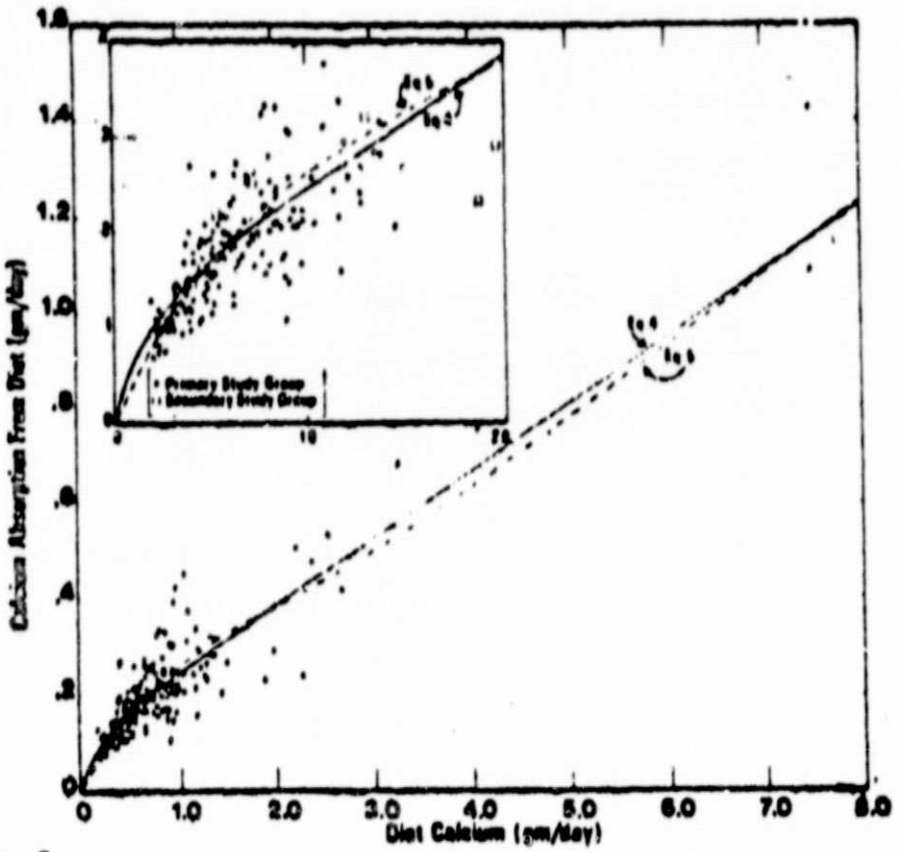

Fig. 5
Plot of calcium absorption from diet as a function of dietary calcium intake. Lines represent least-squares fits of the data to the expressions defined in text. Inset: Detail of lower end of absorption-intake relationship. (31)

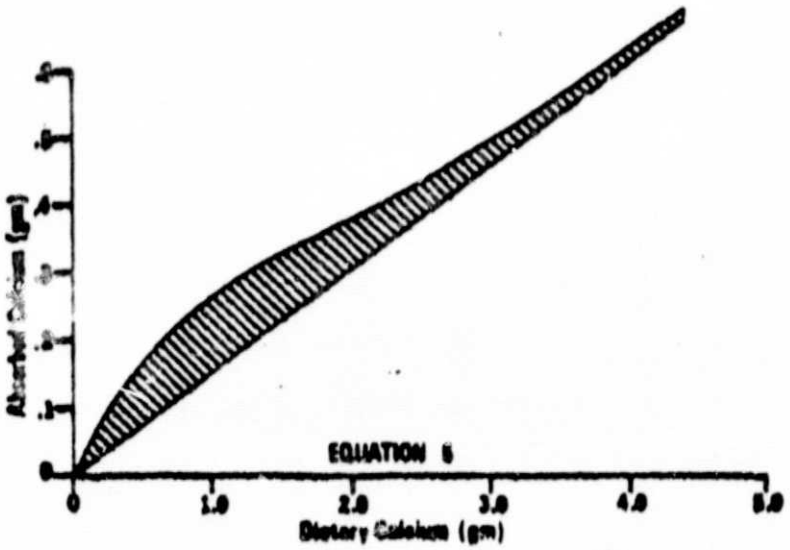

Fig. 6
Graphic comparison of contribution of exponential term of Equations to total absorption. the upper line is the plot of the actual equation, and the lower line, the plot of the linear term only. The shaded area between the two lines represents the absorption component due to the exponential term alone. (38)

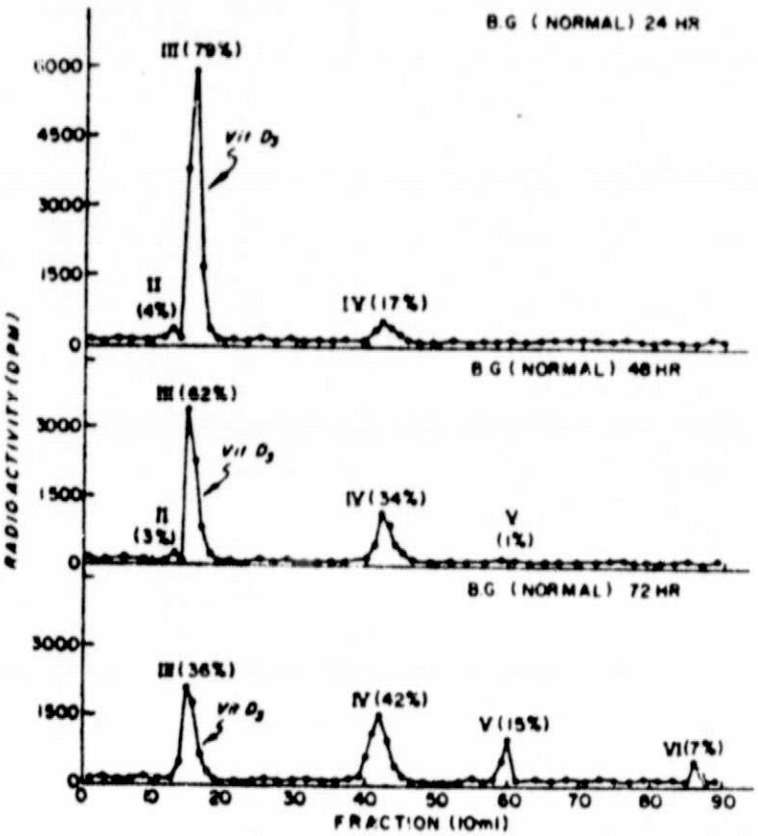

FIGURE 7. Column chromatographic profile of chloroform extracts of 10 ml of plasma obtained in a normal subject 24, 48, and 72 hr after an oral dose of D_2O-^3H.

(From Reference 43)

to the assumptions typical of balance studies also. The problem is reduced when the two routes of administration are studied in succession. In this case, the biochemical and environmental conditions affecting each study must be assumed to be identical. This assumption can be eliminated though by the use of a double isotope method.

With the double isotope method a plasma calcium standard and a simple, precise quantity of oral calcium are established.[52-55] The absorption of calcium is based upon the ratio of the two tracers found in the blood and the urine. An index of endogenous calcium secretion can also be derived from the ratio of injected tracer in the feces. The technique does not depend upon metabolic balance assumptions nor is it subject to the problems typical of balance assumptions. Calcium bone metabolism will not distort the results because stable calcium levels exert no effect. Both tracers are assumed to have identical metabolic characteristics once in the plasma. The results will yield values in terms of fractional absorption of the oral tracer of calcium. If total calcium absorption rates are desired, total calcium ingested must be known, but the total intake of calcium is not essential to the procedure. In a study of weightlessness involving comparisons of pre- and inflight results, calcium intake must follow similar patterns, but again, the calcium intake value is not important, only that the diets are consistent. The procedure is easy and involves the use of ^{45}Ca and ^{47}Ca. Stable calcium isotopes, not abundantly found in nature, such as ^{46}Ca and ^{48}Ca might be used to replace ^{45}Ca and ^{47}Ca, respectively.[56-58] Further evaluation of such a technique must be explored.

This review has briefly described space flight induced osteoporosis, the techniques that were used to detect and measure it, more sensitive techniques that can be used in the future, and possible areas of future examination. The types of issues presented are concerns that need to be discussed and more thoroughly defined to give insight and direction for the development of more informed hypotheses of zero-g induced osteoporosis, of a model of calcium

metabolism, and of paths of research to be taken for the further understanding of space flight osteoporosis. Models are a tremendous aid to the researcher in evaluating his hypotheses, intended research design, and agents to be considered in the research design. A model is capable of directing future goals and proposed research while completed research further refines and evaluates the model. In a complex problem such as the bone demineralization, a wide variety of factors are more easily integrated into the overall process and evaluated. The issues presented in this report are some of the preliminary issues that need to be addressed for the development of a calcium model.

REFERENCES

1. Mack, P. B., P.A. LaChance, G. P. Vose and F. B. Vogt. Bone Demineralization of Foot and Hand of Gemini-Titan IV, V, and VII Astronauts During Orbital Flight. Am. J. Roentgenol., Radium Ther. Nucl. Med., 100(3):505-511, 1967.

2. Mack, P. B. and F. B. Vogt. Roentgenographic Bone Density Changes in Astronauts During Representative Apollo Space Flight. Am. J. Roentgenol. Radium Ther. Nucl. Med., 113(4), 621-633, 1971.

3. Lutwak, L., G. D. Whedon, P. A. LaChance, J. M. Reid and H. S. Lipscomb. Mineral, Electrolyte and Nitrogen Balance Studies of the Gemini-VII Fourteen-Day Orbital Space Flight. J. Clin. Endocr. Metab. 29(9): 1140-1156, 1969.

4. Vogel, J. M., M. W. Whittle, M. C. Smith, Jr. and P. C. Rambaut. Bone Mineral Measurement – Experiment M078. Biomedical Results From Skylab, R. S. Johnston and L. F. Dietlein (eds.) NASA, Washington, D. C. 1977.

5. Rambaut, P. C., C. S. Leach and D. G. Whedon. Weightlessness and Bone Loss in Man, Submitted to Am. J. Clin. Nutr.

6. Leach, C. S. and P. C. Rambaut. Biochemical Observations of Long Duration Manned Orbital Space Flight. JAMWA, 30(4):153-172, 1975.

7. Rambaut, P. C., C. S. Leach and G. D. Whedon. Metabolic Energy Requirements During Manned Orbital Skylab Mission. Life Sciences and Space Research, Vol. XV, 1977.

8. Whedon, G. D., L. Lutwak, P. Rambaut and M. Whittle. Mineral and Nitrogen Metabolic Studies on Skylab Flights and Comparison with Effects of Earth Long Term Recumbency. Life Sciences & Space Research XIV - Akademic-Verlag, Berlin, 1976.

9. Morey, E. R. and D. J. Baylink. Inhibition of Bone Formation During Space Flight. Science (in press).

10. Yagodovsky, V. S., L. A. Triftanidi and G. P. Gorokhova. Space Flight Effects on Skeletal Bones of Rats (Light and EM Examination). Aviat., Space Environ. Med., 47(7):734-738, 1976.

11. Kazarian, L. E. and H. E. Von Gierke. Bone Loss as a Result of Immobilization and Chelation. Clin. Orthop. 45, 67-75, 1969.

12. Hulley, S. B. et al. The Effects of Exercise and Supplemental Phosphate on the Bone Mineral Changes Induced by Bed Rest. NASA Contract ET88567, Final Report, 1970.

13. Vogt. F. B. et al. The Effect of Bed Rest in Various Parameters of Physiological Function, Part XII. The Effect of Bed Rest on Bone Mass and Calcium Balance. NASA Contract E NAS9-1461, Final Report.

14. Rambaut, P. C., L. F. Dietlein, J. M. Vogel, and M. C. Smith, Jr. Comparative Study of Two Direct Methods of Bone Mineral Measurement. Aerospace Med., 43(6):646-650, June 1972.

15. Puumalainen, P. and A. Uimarihuhta. Measurement of Bone Mineral Content Using Gamma-Ray Attenuation by Pair Production. Health Physics, 32: 159-163, 1977.

16. Wahner, H. W., B. L. Riggs, and J. W. Beabout. Diagnosis of Osteoporosis: Usefulness of Photon Absorptiometry at the Radius. J. Nucl. Med., 18(5): 432-437, 1977.

17. Rassow, J. Systematic Errors in Determinations of Bone Mineral Content In Vivo., Symposium on Bone Mineral Determinations, Stockholm/ Studsuik, 1974.

18. Bevan, J. A. Bone Mineral Measurements in the Clinical Situation. Symposium on Bone Mineral Determinations, Stockholm/Studsuik, 1974.

19. Hansen, J. W., G. S. Gordon, and S. G. Prussin. Direct Measurement of Osteolysis in Man. J. Clin. Invest. 52:304-315, 1973.

20. Nelp, W. B., J. D. Denney, R. Murand, G. M. Hinn, J. L. Williams, T. G. Ruad, and H. E. Palmer. Absolute Measurement of Total Body Calcium (Bone Mass) In Vivo. J. Lab. Clin. Med., 79(3):430-438, 1972.

21. Elsasser, U. Quantifizifrung Der Spongiosadighte an Rohrenknochen Mittels Computer Tomographie, Institut Fur Biomedizinische Technik, Zurich, 1977.

22. Ruegsegger, P., U. Elsasser, M. Anliker, H. Gnehm, H. Kind and A. Prader. Quantification of Bone Mineralization Using Computed Tomography, Radiology, 121:93-97, 1976.

23. Exner, G. U., A. Prader, U. Elsasser, P. Ruegsegger and M. Anliker. Bone Densitometry Using Computed Tomography. Part I. Presented in part at the Joint Meeting of the European Society for Paediatric Research, European Society for Paediatric Endocrinology, and Working Group for Mineral Metabolism, Rotterdam, Netherlands, June 1976.

24. Anliker, M. Current and Future Aspects of Biomedical Engineering, Triangle, 16(3/4): 129-140, 1977.

25. Elsasser, U., G. U. Exner, A. Prader and M. Anliker. Dynamics of Trabecular and Compact Bone Mineral of the Radius After Immobilization of the Upper Limb in Children. (To be submitted to Calcified Tissue Research).

26. Palmer, H. E. and M. T. Karagianes. Use of ^{85}Sr as an Indicator of Bone Mineral Replacement in Dogs After Disuse Demineralization. Aviation, Space and Environmental Medicine, 47(11):17-19, 1976.

27. Cohn, S. H., H. Spencer, J. Sahachson and J. S. Robertson. The Turnover of Strontium -85 in Man as Determined by Whole-Body Counting. Radiation Res. 17:173-185, 1962.

28. Eisenberg, E. and J. S. Gordon, Skeletal Dynamics in Man Measured by Nonradioactive Strontium, J. Clin. Invest. 40:1809-1825, 1961.

29. Sargent, T., J. A. Linfoot, and E. L. Isaac. Whole-Body Counting of ^{47}Ca and ^{85}Sr in the Study of Bone Decreases. In Clinical Uses of Whole-Body Counting, International Atomic Energy Agency, 1966.

30. Rosenthall, L. The Role of Strontium 85 in the Detection of Bone Disease. Radiology, 84:75-82, 1965.

31. Spencer, H. Metabolism of Strontium-85 and Calcium -45 in Man. Metab. 9(10):916-925, 1960.

32. Spencer, H. Effect of Magnesium on Radiostrontium Excretion in Man. International J. Appl. Rad. Isotopes, 19, 407-445, 1967.

33. Spencer, H. Factors Modifying Radiostrontium Excretion in Man. Clin. Sci., 17(2):291-301, 1958.

34. Spencer, H. and J. Samachson. Removal of Radiostrontium in Man by Orally Administered Ammonium Chloride Two Weeks After Exposure: The Effect of Low and High Calcium Intake. Clin. Sci. 20(3):333-343, 1961.

35. Spencer, H., I. Lewin, and J. Samachson. Effect of Stable Strontium on Radiostrontium Excretion in Man. Radiation Res. 31:876-888, 1967.

36. Cohn, S. H., S. Spencer, J. Samachson, and J. S. Robertson. Comparative Kinetics of ^{47}Ca and ^{85}Sr in Man. Radiation Res. 19:104-119, 1963.

37. Likens, R.C. Comparative Metabolism of Calcium and Strontium in the Rat. Arch. Biochem. Biophys. 83:472-481, 1959.

38. Heaney, R.P., P.D. Saville, and R.R. Recker. Calcium Absorption as a Function of Calcium Intake. J. Lab. Clin. Med. 85(6):881-890, 1975.

39. Coburn, J.W., D.L. Hartenbo, and A.W. Norman. Metabolism and Action of the Hormone Vitamin D – Its Relation to Diseases of Calcium Homeostasis. West. J. Med., 121:22-44, 1974.

40. Norman, A.W., W.H. Okamura, E.J. Friedlander, H.L. Henry, R.L. Johnson, M.N. Mitra, D.A. Proscal and W. Wecksler. Current Concepts of the Chemical Conformation, Metabolism, and Interaction of the Steroid, Vitamin D, with the Endocrine System for Calcium Homeostasis., Calcif. Tissue Res., 21:153-159, 1976.

41. Parfitt, M., The Actions of Parathyroid Hormone on Bone: Relation to Bone Remodeling and Turnover, Calcium Homeostasis, and Metabolic Bone Disease, Part II, Metabolism 25: 909-955, 1976.

42. Hirsch, P.F. and P.L. Munson, Thyrocalcitonin, Physiol. Rev. 49, 548, 1969.

43. Avioli, L.V., S. Birge, S.W. Lee, and E. Slatopolsky. The Metabolic Fate of Vitamin D_3-^3H in Chronic Renal Failure. J. Clin. Invest., 47:2239-2252, 1968.

44. Avioli, L.V., S.W. Lee, J.E. McDonald, J. Lund and H.F. DeLuca. Metabolism of Vitamin D_3-^3H in Human Subjects: Distribution in Blood, Bile, Feces, and Urine, J. Clin. Invest. :46 (6):983-992, 1967.

45. Heaney, R.P., Radiocalcium Metabolism in Disuse Osteoporosis in Man. Am. J. Med., 33:188-200, 1962.

46. Neer, R., M. Berman, L. Fisher and L.E. Rosenberg. Multicompartmental Analysis of Calcium Kinetics in Normal Adult Males. J. Clin. Invest. 46(8):1364-1379, 1967.

47. Chang, J.M., M. Berman, G.A. Finerman, R.M. Neer, L.E. Rosenberg, and T.J. Hahn, Dietary Perturbation of Calcium Metabolism in Normal Man. Compartmental Analysis, J. Clin. Invest. 48:67-77, 1969.

48. Kales, A.N. and J.M.Chang. Effect of Divided Calcium Intake on Calcium Metabolism, J. Clin. Endocr. 32:83-87, 1971.

49. Kinney, W.R., W.N. Tause and W.H. Dearing. Isotopic Tracer Studies of Intestinal Calcium Absorption. J. Lab. Clin. Med. 66(2):187-203, 1965.

50. Berge, S.J., W.A. Peck, M. Berman, and G.D. Whedon. Study of Calcium Absorption in Man: A Kinetic Analysis and Physiologic Model. J. Clin. Invest. 48, 1705-1713, 1969.

51. Meyer, W.J., I. Transbol, F.C. Bartter, and C. Delea, Control of Calcium Absorption: Effect of Sodium Chloride Loading and Depletion, Metabolism. 25(9):989-993, 1976.

52. DeGrazia, J.A., P.Ivanovich, H. Fellows, and C. Rich. A Double Isotope Method for Measurement of Intestinal Absorption of Calcium in Man, J. Lab. Clin. Med. 66(5):822-829, 1965.

53. Szymendera, J., R.P. Heaney, and P.C. Saville. Intestinal Calcium Absorption: Concurrent Use of Oral and Intravenous Tracers and Calculation by the Inverse Convolution Method. J. Lab. Clin. Med. 79(4):570-578, 1972.

54. Heaney, R.P., P.D. Saville, and R.R. Recker. Calcium Absorption as a Function of Calcium Intake. J. Lab. Clin. Med. 85(6):881-890, 1975.

55. Heaney, R.P., R.R. Recker, and P.D. Saville, Calcium Balance and Calcium Requirements in Middle-Aged Women. Am. J. Clin. Nutr., 30:1603-1611, 1977.

56. Heaney, R.P. and T.G. Skillman. Calcium Metabolism in Normal Human Pregnancy. J. Clin. Endocr. 33:661-670, 1971.

57. Hansen, J.W. and S.G. Prussin, Precise Measurement of Microgram Levels of ^{48}Ca in Biologic Samples by Neutron - Activation Analysis, Int. J. Appl. Rad. Isot. 23, 109-114, 1972.

58. Hansen, J.W., G.S. Gordon, S.G. Prussin, Direct Measurement of Osteolysis in Man. J. Clin. Invest. 52, 304-315, 1973.